REMARKS

Reconsideration is requested.

Claims 1 to 3 and 5 to 18 are pending.

Claim 4 has been canceled, without prejudice.

The applicants affirm the election of Group I, with traverse, for further prosecution in the above.

The applicants respectfully submit the title is appropriate and clearly indicates the invention to which the claims are directed. Accordingly, the Examiner is requested to reconsider and withdraw his objection to the same.

The specification has been amended to include an Abstract, as required by the Examiner.

Claim 14 has been amended to include a period, as required by the Examiner.

To the extent not obviated by the above, the Section 112, second paragraph, rejection of claims 1 to 4, 6, 10, 15 and 16, is traversed. Reconsideration and withdrawal of the rejection is requested in view of the following comments.

The applicants have amended the claims, in part, in response to the Examiner's comments however the Examiner is urged to also consider the following.

With regard to the term "activity", the Examiner is urged to appreciate that the point of the invention is that it provides a way of using recombinant DNA technology in the production of proteins which cannot tolerate other cellular proteins which are essential for host cell activity and cannot simply therefore be deleted. This is clear from the paragraph at the top of page 2 of the application. Many such proteins will have

enzymatic activity but proteins which are essential for cell survival or growth are not limited to enzymes. Therefore, the applicants have amended claim 1 to address the Examiner's concern on the nature of the activity but to also be commensurate in scope with the invention.

As for the term "stable" and "unstable", it is agreed that these are relative terms but the claims, as they presently stand, clearly define the relationship. The precise level of the stability of the polypeptide product is immaterial provided it is more stable than the mutant undesired protein. This relationship is fundamental to the invention and is clear from the claims. The applicants respectfully submit that it would be useless to define these properties in the arbitrary way the Examiner has proposed as this is quite contrary to the spirit of the invention. Similar arguments apply to the term "intact" used in claims 3 and 6, although for the internal consistency of the claims, the claims have been amended to recite "stable".

Claim 4 has been canceled to obviate the objection to the term "elevated temperatures" and claim 5 amended to be made dependent upon claim 3.

In view of the above, withdrawal of the Section 112, second paragraph, rejection is requested.

The Section 103 rejection of claims 1 to 5 and 7 to 17 over Backman (EP 373 962) in view of Kajiyama (U.S. Patent 5,229,285), Liang (Gene 80:21-28), Kiel (Mol Gen Genet 207:294-301), and Belinga (J Chromatogr A 695:33-40), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

Initially, the applicants note the Examiner has combined no less than five references in attempting to assert that the presently claimed invention was obvious. That the Examiner believed five references were required to allege the obviousness of the presently claimed invention is evidence to the contrary.

Moreover, the applicants submit that the principle reference, Backman, describes a process in which highly thermostable enzymes, such as those obtainable from species such as *Thermus aquaticus*, which are intended for use in amplification reactions such as the PCR can be purified. These enzymes, whose extreme thermostability is the feature which makes them targets in the first place, are expressed in a mesophilic cell such as *E. coli* and then purified by heating to extreme temperatures, (80°C for 30 minutes is the exemplified range) in order to denature "contaminants". The contaminants are all other proteins native in the host cell. The cells themselves are selected such that their "proteins generally are denatured at a temperature that does not denature the desired thermophilic enzymes" (see col. 2 lines 45-50). Thus, this describes a fairly crude approach for the purification of proteins which have exceptional thermal stability.

There is no suggestion that the mesophilic cells should be modified in any way, other than to express the thermostable enzyme. Neither would this have been obvious from such a teaching. When one is dealing with enzymes that repeatedly withstand the temperatures found in amplification reactions, it is hardly necessary to consider in great detail the thermostability of potential contaminants from the production process.

The second reference mentioned by the Examiner is Kajiyama et al., which describes a "thermostable luciferase". An essential problem with luciferases is that they are thermolabile, and this means that they are difficult enzymes even to store (see column 1 lines 23-30). Certainly, they are not in the same category as the thermostable enzymes of the type Backman is dealing with. By mutating the luciferase enzyme, Kajiyama finds that the thermostability of this unstable enzyme can be improved, and as the Examiner notes, enzymes can be produced which retain 65% activity following a 50°C incubation for 60 minutes. Although clearly an improvement on wild-type, this is clearly still not in the same league of thermostability as the enzymes of Backman. An ordinarily skilled person would not seriously consider that this level of improvement would mean that the sorts of purification techniques applied in Backman could be applied in this case. Thus, it is not seen that these references would have been combined, absent an impermissible use of hindsight.

Even if these reference were combined, they would still not have led to the invention of the present application.

Liang et al, the third reference mentioned by the Examiner, is concerned with an efficient cloning system utilizing the *recA*$^+$ gene. The point of the work described in the paper is to develop a system which increases the frequency of homogenotization occurring during the cloning process (see page 22 column 1 lines 34-37). Mutant adenylate kinase is used as a model in this transformation system, but no uses for the temperature sensitive mutant are proposed. It is merely an identifiable mutant, which is therefore useful in determining the success of the cloning process.

There is no reason why an ordinarily skilled person would have consulted this reference in conjunction with Backman and/or Kajiyama. The application of a temperature sensitive mutant in a method such as that described in the present application would not have been obvious from a combination of these references, even if there had been motivation to combine the references.

Kiel et al., the fourth reference, does not rectify these deficiencies since it is concerned only with techniques utilized in the transformation of *E. coli* using mutant genes generally. There is no teaching that genes which are unduly sensitive to a particular condition, such as temperature, would be useful in any context.

Finally, Belinga et al teaches that removal of adenylate kinase from luciferase is desirable, but also teaches that this can be achieved using chromatographic techniques. It therefore teaches away from the invention of the present application.

Consequently, it is the applicants' belief that the Examiner's mosaic of the prior art is combined with an impermissible use of hindsight, there was no motivation in the art to combine the cited references and, even if combined, the references fail to teach or suggest the presently claimed invention.

Withdrawal of the Section 103 rejection is requested.

The Section 112, first paragraph, rejection of claims 1 to 6, 10, and 12 to 17 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following comments.

The applicants were the first to appreciate that instabilities that occur in mutant forms of proteins can be used in recombinant DNA technology to allow easier and better purification. This is a very "neat trick" and, once appreciated, can be applied in the production of a wide range of proteins. One merely needs to compare the properties of the target protein and the most troublesome contaminant, and then look for mutant(s) which have differential stability under a particular condition. Contaminant genes having the desired sensitivity or target genes having a particular resistance to certain conditions can then be selected, irrespective of the particular nature of the target protein. These can be done using conventional screening techniques or even utilizing methods such as random mutagenesis as outlined in the final paragraph on page 4 of the application.

It is probable that one would only need to go to these lengths in cases where purification of a particular protein is unduly difficult using conventional methods. Nevertheless, such situations are likely to appear in many fields, not purely in luciferase production. There is no reason why these methods could not be applied broadly.

The Examiner has suggested that the claims encompass the separation of proteins under any conditions. This is incorrect. It is clear from claim 1 that the conditions used must be appropriate to destroy the undesired protein, but allow the target polypeptide to remain intact. With the advanced state of biotechnology at the time of the present invention, and the library screening procedures available, the level of experimentation which would have been required to allow an ordinarily skilled person to go through the selection process in any particular case, would not have been undue.

The applicants submit that the claimed invention is pioneering in nature for the reasons discussed above, and that therefore the applicants are entitled to broad protection. Consequently, the Section 112, first paragraph, rejection should be withdrawn.

In view of the above, the claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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MARKED UP CLAIMS

1. (Amended) A method for producing a polypeptide product which is substantially free of an undesired protein which is a protein whose activity is essential for survival or efficiency of a host cell, the process comprising culturing a host cell which is able to express said polypeptide product and which is able to express said undesired protein only in a mutant form which form has the activity of the corresponding native protein under culture conditions but is unstable under conditions at which the said polypeptide product remains stable; and recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to conditions under which the undesired protein is unstable so as to denature the undesired protein.

3. (Twice Amended) A method according to claim 1 or claim 2 wherein the conditions at which the undesired protein is denatured and the polypeptide product remains stable [intact] are temperature conditions.

5. (Amended) A method according to claim [4] 3 wherein the elevated temperature is 37°C or more.

6. (Twice Amended) A method according to claim 1 or claim 2 wherein the conditions at which the undesired protein is denatured and the polypeptide product remains [intact] stable are pH conditions.

10. (Amended) A recombinant cell which comprises a first nucleotide sequence which encodes a desired polypeptide under the control of regulatory elements which allow expression of said polypeptide, and wherein a gene which encodes a protein which is undesirable as a contaminant in preparations of said polypeptide product but whose activity is essential for survival or efficiency of a host cell, is mutated such that

the protein expressed is unstable under conditions in which the polypeptide product remains stable.

14. (Amended) A recombinant cell according to claim 13 which comprises a recombinant *E. coli* cell.